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THE PHYLLOSPHERE

I. AN ECOLOGICALLY NEGLECTED MILIEU

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INTRODUCTION

In an ecological analysis of the habitat of epiphytes, carried out in Java, Indonesia, attention was drawn to the rhizosphere of the epiphyte [42]. Observations were made on the root systems of the fern *Drymoglossum pilloselloides* Pres land some orchids, all commonly occurring on the trunks and branches of trees, but often growing on leaves. In addition to the symbiotic root-inhabiting fungi a number of other, mostly saprophytic, soil fungi and fungi regarded as weak pathogens could be isolated. A few characteristic colonies of yeasts and bacteria appeared regularly. *Cryptococcus* and *Beijerinckia* species, and *Mycoplana rubra* De Vries and Derx were obtained in cultures.

The occurrence of soil bacteria in such situations is not surprising. The root system of epiphytes is a natural trap for air- and water-borne micro-organisms and the reaction (pH 5.5) of the medium used for the isolation of the fungi is favourable for the growth of the epiphyte rhizosphere bacteria. Beijerinckia is abundant in acid tropical soils. Thus, it is not astonishing that the humus-rich substrate, formed by the decomposition of leaf litter and senescent and sloughed-off periderm cells of the trees on which the epiphytes grow, provides a suitable environment for Beijerinckia. Moreover, the foliar exudates which are washed down the crevices of the bark provide a supply of nutrients sufficient to maintain a complex community.

Extending this consideration to the actual source, the foliage, of this downward nutrient stream, it is similarly obvious that the

leaf itself, provided that it is mechanically strong enough and has a sufficient length of life, should be able to support the growth of epiphytes. Dust and plant debris collect between the roots of such leaf epiphytes and these in turn would provide an environment for micro-organisms.

As a preliminary attempt to investigate whether the occurrence of these micro-organisms is dependent on the rhizosphere of the epiphyte, comparative platings were made from the epiphyte root system and an adjacent area of the supporting leaf, which was not covered by the epiphyte.

In a preliminary note [43] the author has reported the preponderance of fungi over bacteria in the rhizosphere, a condition which persisted throughout the various stages of development of the supporting leaf. On the other hand the initial predominance of bacteria on leaf surface not occupied by the epiphyte alters with age, so that on senescent leaves fungi apparently predominate. The microbial population, as determined by the use of Burgeff's acid medium, appeared to have a characteristic composition and to contain a limited number of species, with *Beijerinckia* sp. as the characteristic bacterial representative.

The next step was to ascertain whether these organisms could also be isolated from healthy leaves not bearing epiphytes. This proved to be the case. Beijerinckia was isolated in 192 out of 196 cases examined.

The external surface of the leaf, as an environment for micro-organisms, has been termed the "*phyllosphere*" by analogy with the rhizosphere of roots (Ruinen [43]). Based on similar considerations Last [29] suggested independently the same term as a result of a study of the incidence of fungi on cereal leaves. Since then, the term has been accepted (*e.g.* Di Menna [38], Greenland [17], Kerling [27]).

So far, with a single exception, the occurrence of Beijerinckia has been reported to be pantropical. Kluyver and Becking [28] pointed to the prevalence of this bacterium in lateritic soils as a possible explanation of its limited distribution, which, however, is not completely satisfactory. Derx [12] suggested a possible association with the roots of specific tropical vegetation, presumably leguminous plants or rice.

The observation, however, that the leaf surface of a great number

of plants growing in humid tropical conditions offers a suitable habitat for micro-organisms in general and for Beijerinckia in particular still offers another explanation of the widespread occurrence of this bacterium. The probable importance of the nitrogen-fixing properties of Beijerinckia for this environment, justified a further investigation of the phyllosphere. The primary aim of this investigation was to ascertain whether Beijerinckia and, possibly, other nitrogen fixing bacteria occur in the phyllosphere and are characteristic for this habitat. Moreover, it seemed desirable to find out which environmental factor or factors condition this habitat.
The survey made in Indonesia in 1955 was extended during an eight-months stay in Surinam at the Government Agricultural Station, Paramaribo in 1956–1957. In this paper the preliminary findings are presented with a discussion of the problems arising.

MATERIALS

The material consisted of fresh leaves collected at random in different localities of Java, Sumatra, Banka, and Bali. They were picked from trees up to 24 m in height, climbers, shrubs, perennials, and epiphytes growing in the Bogor Botanic Garden, in primeval and secondary forest, and from coastal vegetation and plants growing in craters. At first similar random sampling was carried out in Surinam, material being obtained from wayside and jungle vegetation of the forest floor as well as from the crown of felled trees. Only a part of this botanical material could be identified taxonomically. It contained ferns, along with monocotyledonous and dicotyledonous leaves. Later the leaves of citrus, cacao, and shade trees growing in the experimental plots of the Agricultural Station were selected for a closer study.

In the first instance healthy-looking mature leaves were sampled. Later young and senescent leaves were also included in the observations.

METHODS

The presence and the extent of the phyllosphere population has been ascertained *i.* by direct microscopy and *ii.* by selective culture in nitrogen-free media.

i. Direct microscopy

Microscopical examination of free-hand surface sections of the fresh leaf at medium (400 ×) or high (800 to 1000 ×) magnification is easy and fully satisfactory. Staining with phenolic erythrosine or Lugol's iodine solution increases contrast and reveals lipid drops in the organisms and the environment. A disadvantage of this method is that during preparation of the section

part of the microbial population may be lost due to swelling and disintegration of the microbial layer of the phyllosphere. Much depends on the species composition of the microbial population and the weather conditions immediately prior to the sample leaves being taken; a recent or prolonged wetting increases the chances of loss of microbes.

A more attractive method, which is also suitable for the preparation of permanent mounts necessitates infiltration of the microbial layer of the phyllosphere with a solution of collodion or, even better, a solution of water-soluble plastic material. When dry the film is stripped off and mounted for examination. Sometimes part of the microbial layer remains attached to the leaf, particularly so if the layer is thick or anchored by fungal hyphae which have grown into the epidermis. Comparison of a number of preparations yields a satisfactory over-all picture of the composition and development of the microbial layer of the phyllosphere.

ii. Selective culture in nitrogen-free media

a. Sterile, powdered starch scattered lightly over the surface of leaves lying in petri dishes was slightly moistened and the leaves then incubated at room temperature in an atmosphere saturated with water vapour. If microscopical examination after 24- or 48-hours incubation revealed the presence of bacterial cells between the starch grains, samples were plated out on glucose–phosphate or starch agar having a reaction of pH 5.5 for Beijerinckia and pH 7.0 tp 7.2 for Azotobacter. By repeated replating the organisms could be isolated in pure culture.

b. The leaves were immersed in a shallow layer of liquid medium, containing 1% glucose + 0.1% KH_2PO_4 or 0.05% K_2HPO_4 in 250-ml flasks. The former medium yields mainly Beijerinckia, the latter the common Azotobacters. After a few days incubation the various organisms were isolated by the use of agar plates.

At a later stage of the survey in only a few cases selective culture was carried out by the method devised by Derx [10,11], and also used by Jensen[26], which makes possible the simultaneous detection of the various *Azotobacter* species occurring in a sample. The liquid media used had the following composition:

0.1% KH_2PO_4 + 1% glucose	*Beijerinckia* species
0.05% K_2HPO_4 + 1% ethanol + 0.1% $CaCO_3$	*Beijerinckia* spec., *Azotobacter insigne, A. agile, A. vinelandii, A. chroococcum* and others
,, + 1% mannitol + 0.1% $CaCO_3$	*A. vinelandii, A. chroococcum*
,, + 1% sodiumbenzoate + 1% ethanol + 0.1% $CaCO_3$	*A. agile*
,, + 1% sodiumbenzoate + 0.5% mannitol + 0.1% $CaCO_3$	*A. vinelandii*

Incubation at room temperature.

RESULTS

General considerations

The findings in Surinam fully confirm those made previously in Indonesia. Well-developed phyllosphere populations are of general occurrence. In addition to Beijerinckia a number of oligonitrophilic and nitrogen-fixing micro-organisms could be isolated by the use of media having a reaction near neutrality. Among these are a number of yeasts and fungi, along with species of *Aerobacter*, *Arthrobacter* and *Pseudomonas*. The presence of these phyllosphere organisms is visible with the naked eye as a patchyness or as a hard gloss on the surface of the epidermis proper, depending on their distribution in scattered groups or in a continuous film. In addition to the mentioned organisms a highly complex population, which often gives a specific colour to the phyllosphere, is usual. Thus, a black or blackish phyllosphere colour will point to the presence of Cyanophyceae or sooty moulds, whereas patches with light bright-green or reddish tinges point to Chlorophyceae, and greyish colours to moulds. The bluish green, white-bordered patches of crust lichens are most conspicuous. Beneath the microbial layer the leaves are dark green unless they are, incidentally, infected by parasites.

The microbial layers of the phyllosphere show marked differences in species composition and surface spread, which are seemingly characteristic for particular plant species. No further attention has, sofar, been paid to this aspect, which offers subject for further investigation.

Not only do different plant species show striking differences in their phyllosphere populations, but different leaves of one and the same plant show variations depending on the position of the leaf in the vegetation and on the age of the leaves. As a rule the increase in population starts after the leaf has reached maturity and may reach considerable dimensions on the older leaves (Plates 1, 2, 7, 8, and 9).

In view of the changes in the population as the phyllosphere layer develops, it is easily understandable that the enrichment cultures give different results for different sample leaves. Particularly in the case of older leaves, samples removed within 24 hours of the commencement of incubation has a flush of bacteria which could obviously grow more rapidly on the combined nutrients and the

added substrate than the Azotobacteriaceae, and were therefore, more easily purified. After prolonged incubation of the leaves (2 to 5 days) in the presence of the nutrient medium *Azotobacter* spp. and *Beijerinckia* spp. appeared. On the other hand, in the case of young mature leaves, the latter species could be found to have multiplied abundantly after eight hours of incubation.

The micro-organism population of the phyllosphere

It has been mentioned already that during the survey of the phyllospheres of various plant species in Indonesia, Beijerinckia was found in 192 of 196 leaf samples. The four cases in which Beijerinckia was not found seem comparitively insignificant. They may be due either to faulty manipulation or to the absence of this organism from the phyllosphere population of these particular leaf samples. That an incubation time of 24 hours is either too long or too short for the various kinds of micro-organisms became obvious during the course of the survey. On the other hand the acid reaction of the medium used for the isolation of Beijerinckia may have precluded the development of, for instance, Azotobacter, which, as became apparent later, often form the main component of the phyllosphere population.

In Surinam, a preliminary indication of the presence of potential nitrogen-fixing bacteria in the phyllosphere was obtained by incubation of the leaves in media having either an acid or neutral reaction and to which 1% glucose has been added. Selective cultures in accordance with the method used by Derx were subsequently set up in the case of a few leaf samples. Microscopical examination of the leaf surface was carried out in all cases. These observations covered a wider range of micro-organisms, and a smaller number of higher plant species.

Both direct microscopical examinations and culture methods provided information about the other inhabitants of the phyllosphere community. The data presented below are incomplete as only those organisms which could be readily identified were recorded. Moreover, at high magnifications (800 to 1000×) only fractions of the total leaf surface could be examined and, although an over-all picture could be formed, many details will certainly have escaped observation. Furthermore, it should be remembered that the main object of the investigation was to ascertain the occurrence of nitro-

gen-fixing bacteria in the phyllosphere. These, therefore, received most attention. In addition the culture media used were not suitable for the growth of organisms other than those having minimum requirements for combined nitrogen. Consequently, it does not necessarily follow that the organisms appearing in the table were the only ones initially present in the sample.

The observations made in Surinam are summarized in Table 1. For tabulation the organisms have been separated into groups, *i.e.* bacteria, actinomycetes, fungi, and algae; the bacteria being separated into genera and species in the case of some of the Azotobacters,

TABLE 1

Organisms found by selective culture and direct microscopical observation in the phyllosphere of plants in Surinam

Origin of samples	Number of samples	Bacteria										Actinomycetes	Fungi		Algae		Other organisms		
		Clostridium spp.	*Beijerinckia* spp.	*Azotobacter* spp.	*A. chroococcum*	*A. vinelandii*	*A. agile*	*A. insigne*	*A. beijerinckii*	*Aerobacter* spp.	*Spirillum* spp.		Yeasts	Fungi	Chlorophyceae	Cyanophyceae	Lichens	Mosses	Prozotoa
Random samples of leaves from virgin forest	15	4	15	14	4	7		2		9	11	15	9	15	8	2	3		11
Random samples of leaves from trees in experimental gardens	12		10	11		1			1	2	2		7	12	3	9	7		2
Random samples of Tillandsia leaves	2		2	1					1		2		1	2		1			
Random samples of Citrus leaves	24	1	8	24	8	3			2	6	8	2	8	20	14	7	9	1	2
Random samples of Cacao leaves	12	1	11	12	3	2	1				8	1	8	12	12	6	2	1	6

isolated according to the method of Derx, the remainder being enumerated simply as yeasts, fungi, Chlorophyceae, Cyanophyceae, lichens, mosses, and protozoa. A great part of the organisms is highly motile. A detailed report of the findings will be published elsewhere.

Development and extent of the phyllosphere micro-organisms population

It is evident that during the development of a leaf micro-organisms will be absent from the leaf surface for only a short time, if at all. In an established plant community the presence of an abundant phyllosphere population of micro-organisms precludes the absence of micro-organisms on the surface of the developing leaves. Actually, bacteria and hyphal fragments have been observed even on leaves at the earliest stage of development; these are obviously the result of infection via rain spattering or dew dripping from leaves at a higher level (Plate 3). The nutrients in this water are sufficient for only a limited number of divisions of these microbes. Vigorous development of the organisms occurs during the maturity of the leaf when it is photosynthesizing and transpiring actively.

At this stage competition between the various types of organisms in the inoculum commences. At first the nitrogen-fixing organisms are in a favourable position since they are independent of combined nitrogen and not exacting as to the source of energy. They have been observed to multiply in the capillary water held by surface structures, *e.g.* trichomes, depressions in the epidermis overlying the anticlinal cell walls, and later in the vicinity of fungal hyphae where nitrogen is, or becomes, insufficient for the growth of the latter (Plate 3 to 7).

Plate 3 shows one of many minute colonies found on a young Qualea leaf from the canopy of virgin forest after a recent infection. In the drop of fluid a small colony of cocci has developed, whereas there has been no comparable development of bacilli. In the case of an older leaf (Plate 4) secondary colonies and hyphae have developed on top of the continuous layer of bacteria covering the surface. In Plates 5 and 6 of *Coffea* sp. and *Pellionia* sp., respectively, the epidermal cells are clearly outlined by localized growth of micro-organisms in the depressions. This can be considered an indication of the stage at which exudation from the leaf commences. After this stage the micro-organisms multiply actively and cover the entire leaf surface, and the layer subsequently grows in thickness.

Eventually the nitrogen-fixing bacteria will supply the necessary combined nitrogen for the development and maintenance of the other members of the phyllosphere population. The physiological state of the supporting foliage will ultimately be the factor determining which of the micro-organisms will become dominant in the phyllosphere.

Plate 1. Cacao leaves of various ages. On the left a recently mature "clean" leaf; on the right a senescent leaf with conspicuous patchy growth of lichens and with algal growth along the veins. The leaves between show intermediate stages.

late 2. Surface section of a leaf of *Eria* sp. from Sumatra. At *a* the epider-
is is covered with the phyllosphere population, at *b* the leaf epidermis is
re, at *c* the microbial layer is hanging free in the preparation. In the micro-
bial layer yeasts, fungi, and algae are visible in the binding mass of
Beijerinckia cells (see insert) (300×; insert 700×).

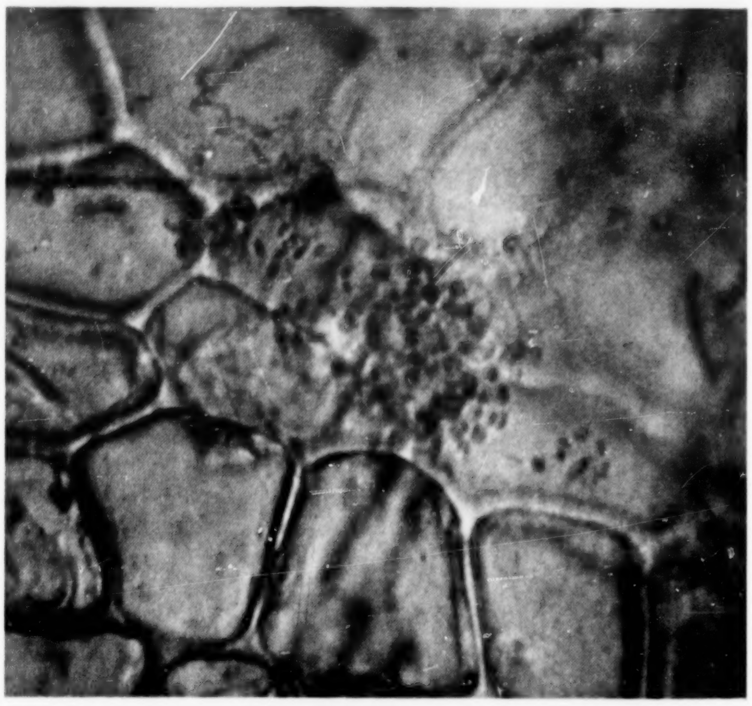
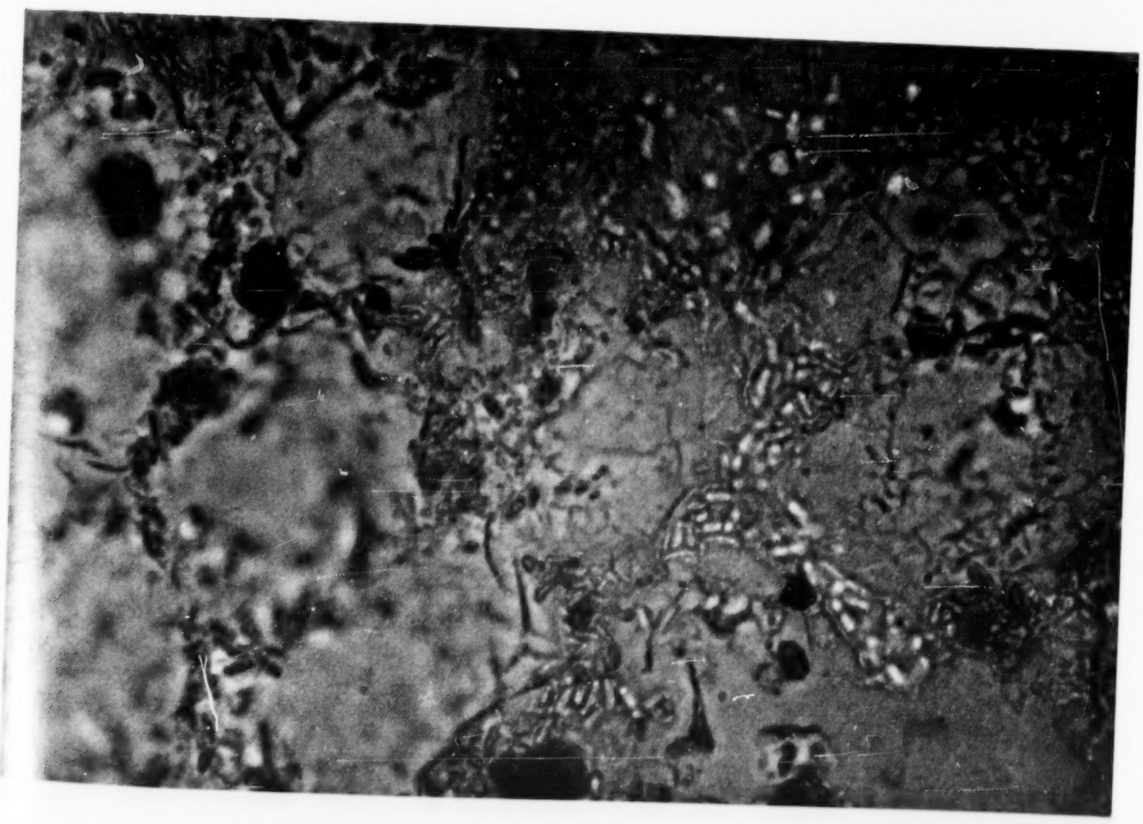


Plate 3. Appearance of the surface of a young leaf of *Qualea* sp. from the canopy of the Surinam virgin forest. Initial development of diplococci in a primary mixed colonization in a well-contoured drop of dew (700×).

Plate 4. The same as Plate 3, but at a later stage. On the surface of a thin but continuous layer of bacteria secondary micro-organisms have developed in flush in drops of water and in capillary water along the hyphae (525×).

Plate 5. Appearance of the surface of a young mature leaf of *Coffea* sp. from Java. Microbial growth, mostly *Beijerinckia* spp. along the depressions over the anticlinal walls of the epidermal cells (700×).



ate 6. Surface of leaf of *Pelliona* sp., a common plant of the forest floor West Java, from a plant grown in the greenhouse of the Leiden Botanic rden. Growth of micro-organisms; Azotobacter, *Beijerinckia* sp., actinomy-es, and yeasts spreading from the depressions over the anticlinal walls of epidermal cells over the intervening projections of the cells (700×).

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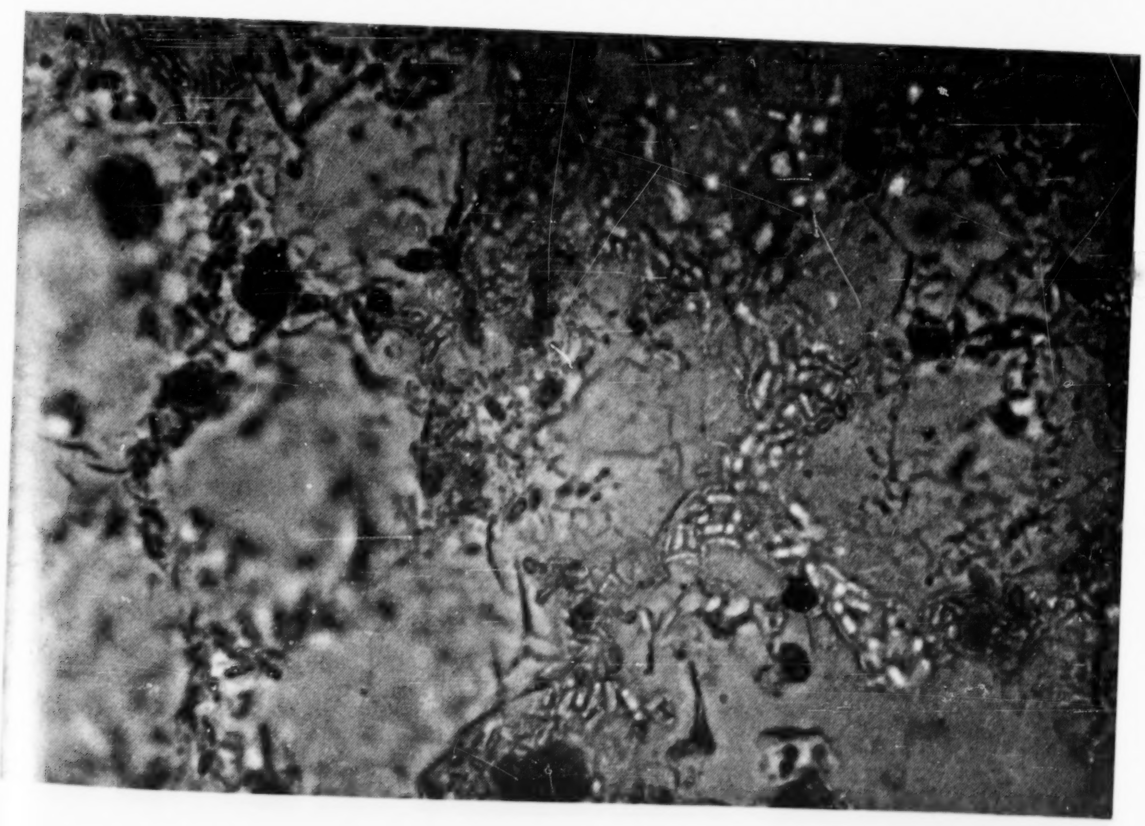

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Plate 7. Collodion film with embedded elements of the phyllosphere po-
pulation from a mature leaf of *Citrus* sp. from Surinam. Only part of th
population has been removed in the film. In the upper left hand corner th
impression left by bacteria and hyphae on the surface of the film can b
seen. In the centre a patch of algal growth can be seen on top of the lay
of Azotobacter (*a*), *Trentepohlia* sp. (*b*), and a young thallus of Phycopelt
(*c*). There is a net of fungal hyphae and a scattering of pollen grains over th
whole surface, a fungus sporangium can be seen at *d* (525 ×).

In the specimens examined the microbial layer covering the leaf surface varied from 1 to 22 μ in thickness, the projecting filaments of algae, fungi, and lichens not included.

It is frequently considered that the magnitude of the bacterial population is an important indication of the fertility of the soil. For comparison of phyllosphere populations with soil populations counts of bacteria on the leaf surface of a mature cacao leaf were made.

The leaf sample was collected in the Government Experimental Gardens in Paramaribo after a period of continuous rain (31 December 1956 6.5 mm; 1 January 1957 61.2 mm). Loss of bacteria during preparation of the material could not be prevented due to the water-logged state of the microbial layers.

In addition to a well-developed hyphal net larger organisms were present, *e.g.* Chlorella and Scytonema. The bacteria filled the spaces in the network and were concentrated in thick clusters round the algae (Plate 8). The counts were made at some distance of the algae, where the bacterial layer was only two or three cells thick. The estimate obtained may therefore be expected to be on the low side.

The density per square of $8 \times 8\ \mu$ varies from 6 for the larger Azotobacters to 17 for Beijerinckia. Taking a mean of 8 bacteria per square the population amounts to 12.5 millions per square centimeter *.

The ready appearance of a number of oligonitrophilic organisms within 24 hours after inoculation of a nitrogen-deficient medium with leaf material suggests that these organisms are either actively growing or in a quiescent condition on the leaf surface, such that they respond readily to favourable changes in the environment. In this connection the following observation seemed remarkable Moisture collected in the morning from cacao leaves in Surinam was highly viscous, turbid and of a yellowish green colour. Microscopical examination showed it to be teeming with highly motile organisms (bacteria and some flagellates), and with less conspicuous microbes (yeasts and algae). Inoculation of this fluid at a dilution 1 : 1000 into an ethanol–phosphate medium gave rise to a

* Compared with Azotobacter counts for fertile soil of the temperate regions (from 50 to 100 thousand/gram soil) and with the computed total microbial numbers per gram of soil in tropical forest, *e.g.* 500,000 in Malaya (Corbet [8]), 2219 to 2820 millions in Kenya (Meiklejohn [37]) or 2,860,000 in Brazil in a citrus plantation (Döbereiner [13]) the figure is impressive.

yellowish gray turbidity within four hours, due to bacteria, some yeasts, and flagellates. Direct examination of the leaf showed the striking development of a multi-layered population of micro-organisms on the leaf surface. It has to be mentioned that the sample had been collected after two days without appreciable rainfall, *i.e.* 1.7 and 0 mm, respectively, and with heavy dew during the nights.

Observations on other leaves of the cacao and of other plant species yielded similar results.

As has been mentioned before, the kinds of micro-organisms occurring in the phyllosphere populations and the degree to which the leaf surface is covered vary for different plant species. Cacao leaves from the experimental plot of the Agricultural Station at Paramaribo appeared to be more readily colonized by Beijerinckia than by the species of Azotobacter, whereas the latter apparently predominate on citrus leaves. Re-examination of cacao and citrus leaves from plants growing in different localities is needed since such differences in the predominant species may well be as much the result of an initially heavier specific infection, as the result of a selective habitat offered by a specific plant, either as the consequence of its nutritional status or its specific exudates. Moreover, the location within a particular plant community proved to be of importance.

Along with the increasing excretion of substrate material from the leaf at the time when the leaf reaches maturity nutritional conditions for colonization improve and other micro-organisms follow the pioneers. The release of the metabolites and autolysis products of these organisms further influences the nutritional environment. Plate 1 shows the gradual colonization of the surface of a cacao leaf in which the larger elements such as algae are becoming increasingly conspicuous.

Summarizing the findings from direct observations and from cultures it appears that the following sequence occurs (see Fig. 1).

The phyllosphere is first colonized by bacteria, actinomycetes, and fungi. When colonization commences, the oligonitrophilic bacteria have an advantage due to their less exacting nutritional requirements. Moreover, many of them are motile and they spread rapidly over the leaf surface when it is wet. The nutrients supplied

by the leaf exudates and by the Azotobacter population provide an adequate substrate for successive colonizers; algae, yeasts, and lichens appear and a mixed population of other unicellular organisms such as flagellates, amoebae, myxomycetes, and ciliates develops, which feeds on the other micro-organisms. Arthropods may follow in due course.

This is generally the end of the succession. Under conditions of adequate humidity, temperature, nutrition and longevity of the leaf, however, mosses, ferns, and occasionally phanerograms, well-known epiphytes, may develop. Such a much expanded phyllosphere population presents conditions for colonization by still more micro-organisms in the tangle of roots and on the leaflets of the epiphyllic vegetation. A rhizosphere population will now develop as a result of the development of this new vegetation and the bacteria will colonize the additional leaf area and occupy the phyllosphere of these later immigrants.

The climax population of the phyllosphere as described above will be reached and maintained on the mature and fully active leaf. Later, and especially as metabolism of the leaf and exudation into the phyllosphere decrease during senescense, the microvegetation will gradually become more autonomous. The nutritional requirements will be met by mutual exchange between the various organisms of the phyllosphere and from leachings from leaves situated at higher levels in the plant community. At this stage the leaf functions chiefly as a mechanical support.

It may be mentioned that the juvenile stage of the leaves lasts for only a couple of days up to a week for many species, whilst the

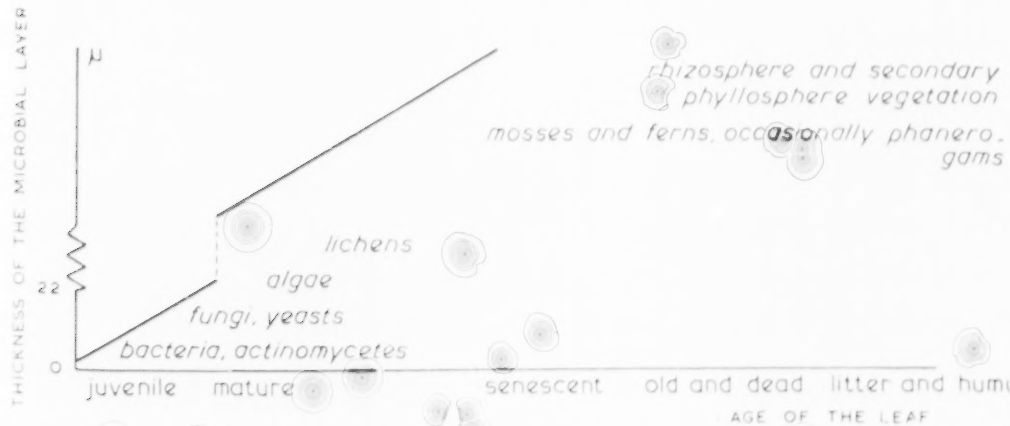


Fig. 1. Development of and sequence in the phyllosphere population during growth and senescence of the leaf.

period of maturity may be extended in many non-deciduous species up to a couple of years.

The above scheme representing the conditions prevailing during the development of the phyllosphere population is suggested.

The phyllosphere as an environment

After it had been ascertained that microbial growth occurred in great, although variable abundance on the leaf surface the next step was to look which conditions favour the development of the micro-organisms and make the phyllosphere, as it is by definition, an environment particularly suited for microbial life. It proved to be a typically aqueous millieu which is continually fluctuating in volume and in nutrient content.

In the foregoing paragraphs the role of the water has been mentioned already. It offers the life space as well as the medium of exchange with the leaf and the other members of the community. Its quantity, the time of day, and the duration of the wetting all have a considerable influence upon the growth of the micro-organisms, and the composition of the population. Obviously stagnant fluid, *e.g.* in the form of drops of dew, causes an increase in the population which is strictly confined to these drops. As has been mentioned before a damask-like texture is produced in the phyllosphere, due to well-localized, slightly thicker layers of organisms after the evaporation of the water and its absorption by the leaf. On the other hand, rainwater, which spreads evenly over the epidermis produces a microbial layer of uniform thickness; when dry this layer has the glossy appearance of hard lacquer, and is often mistaken for the leaf cuticle. Drainage of the water along the veins causes an accumulation of organisms, also visible with the naked eye (Plates 1, 9).

The variable effect of the water is most clearly observed in the different strata of the green vegetation. The leaves exposed to the sky and prone to the direct wetting by dew and rain show the above sketched features. However, the parts of the leaves which are overlapped by other leaves, as is often the case in large-leaved species, lack the specific gloss or patchyness, even in the outer layers of the vegetation. The differences are still more pronounced beneath the canopy there where during the dry season direct wetting is irregular and occurs only occasionally by the drip of rainwater,

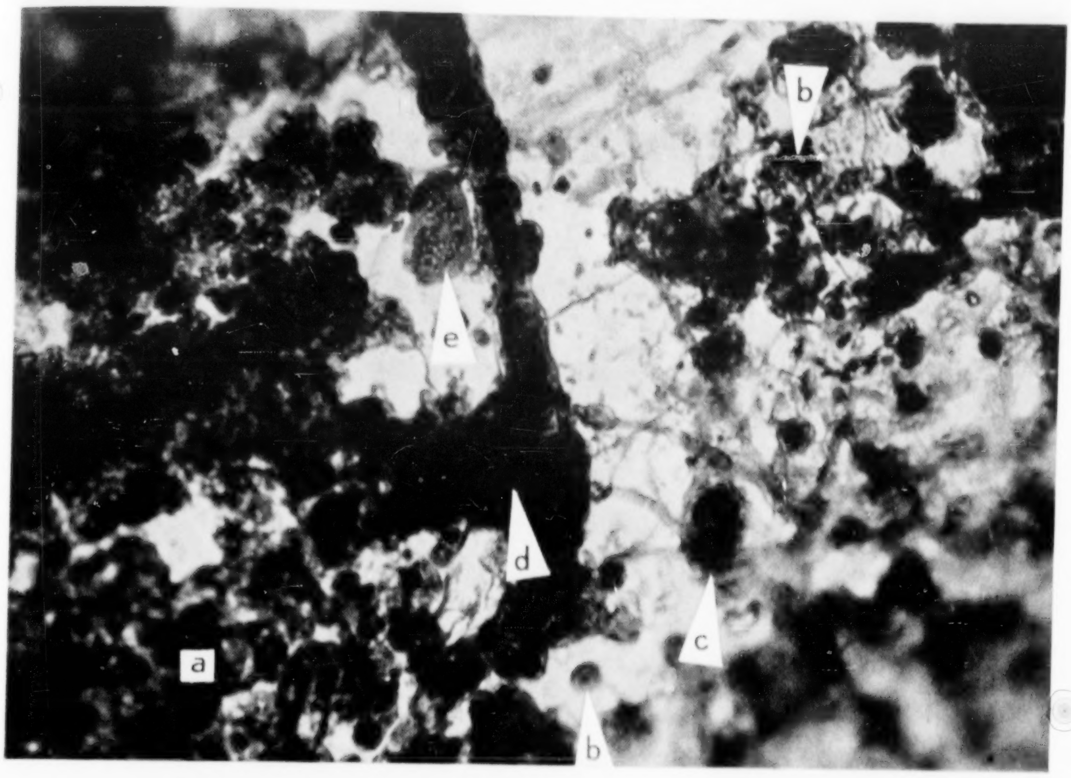
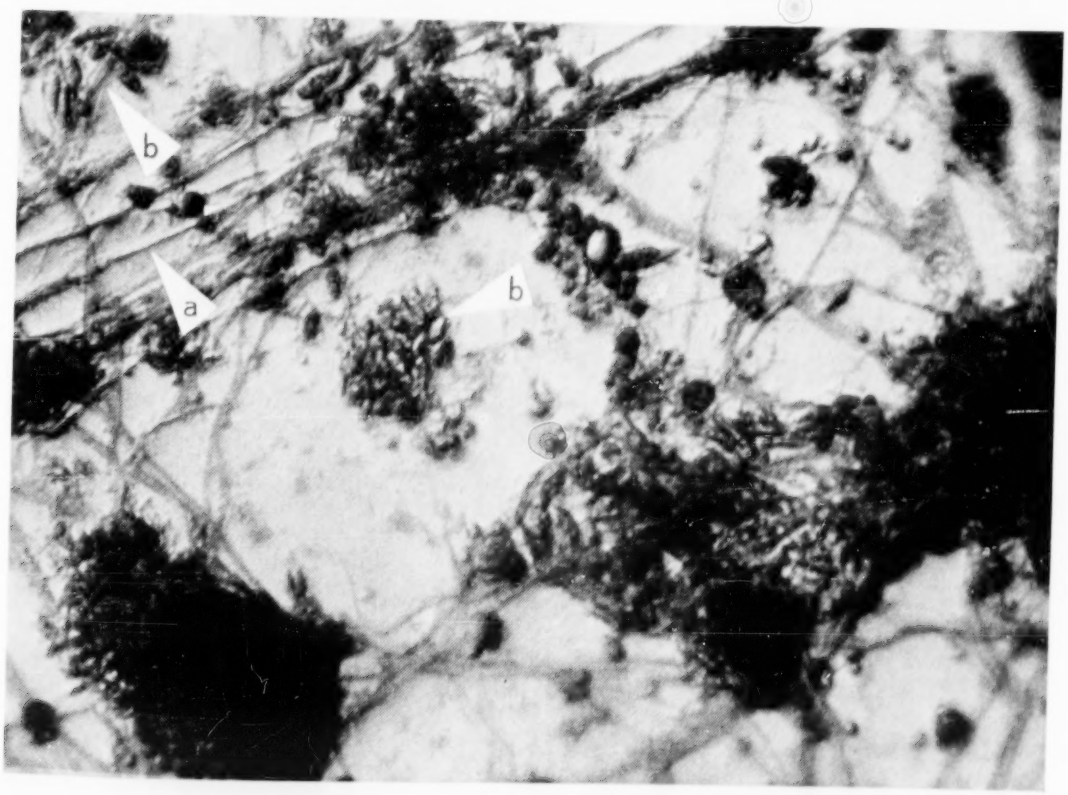


Plate 8. Part of phyllosphere population from surface of mature cacao leaf from Surinam embedded in collodion film. The following organisms can be observed. Groups of bacteria (*a*), yeasts and unicellular algae (*b*), and hormogonium of a lichen (*c*). In the centre of the photograph a filament of Scytonema (*d*) and a protozoon (*e*) can be seen (525 ×).



ate 9. Hyphae growing in the depression above a vein of a cacao leaf (*a*). The masses of ellipsoid cells at (*b*) are unicellular algae (700 ×).

and dew from the higher levels of the vegetation, or by condensing water vapour from the soil. The resulting microflora, with a preponderance of hyphomycetes and lichens is indicative for the microclimate of high relative humidity but irregular wetting. In the places where a regular run-off from the higher strata occurs, a completely different and more hygrophilic micro- and macrovegetation develops. Many of the organisms concerned have a high rate of division and a light dew suffices for active multiplication to take place (*cf* p. 88). The organisms which divide more slowly lag behind, especially so, if the wetting is of short duration, as is often the case with rain falling during the day. In Indonesia as well as in Surinam locally wetting by dew and ground mist during ten to twelve hours a day is common. In the early morning the vegetation is dripping wet and in the shade the dew remains visible till ten o'clock a.m. Relevant figures about the seasonal incidence and the duration of wetting have not been gathered, as the influence of the water on the accumulation of the phyllosphere organisms only became clear during the course of the observations. They are subject for a future research.

The wettability of the epidermis proved to be another important factor in the development of the phyllosphere population, which may be due to the transfer of nutrients from the leaf to the phyllosphere, and *vice versa*. The fluctuation in this property in relation to the age of the leaf is a common feature. It has been noted by the author that the young leaves of many tropical plants pass from a stage where they are easily wetted and readily absorb water, through a short stage of complete unwettability, which precedes maturity, to a stage of increasing wettability again. In the initial stage close-standing trichomes, as *e.g.* on cacao leaves, hold the water capillary. The wettability post maturity seems to be more complex and may be associated with the weathering by atmospheric conditions and the activity of micro-organisms. The ectoplasmodesmata, which were found to be microscopically visible in many leaves, may be involved in the processes.

The species composition of the phyllosphere population, and the climatic conditions in existence prior to the wetting, influence the uptake of water. Thus, algae and bacteria increase whilst fungi and lichens decrease wettability. The excretion of lipids by the latter

micro-organisms plays a role in determining the surface run-off at the start of rain.

The nutrients in the phyllosphere are furnished by the green plants in the form of foliar exudates. They contain mineral and organic compounds, the latter being characteristic for the different plant species. At a later stage the metabolic and autolysis products of the developing phyllosphere population contribute to the complexity of the composition and the fluctuations in the nutrient medium, whereas sucking insects by their excrements and by stimulation of the excretion of organic material from the leaves cause a further increase.

In the short time available in Surinam only a few analyses have been carried out on the mineral content of dew on cacao leaves. Here the level of potassium appeared to lie at about 80 mg per litre; of total and dissolved nitrogen at 45.5 mg and 0.84 mg per litre, respectively; of total and dissolved phosphorus at 26 mg and 0.36 mg per litre. It is evident that the first figures represent for the larger part the nitrogen and phosphorus locked up in the phyllosphere population. No observations have been done in Surinam on the organic matter present on the surface of the leaf. Analyses made by the author in Holland show that *e.g.* sugars and organic acids are present on the leaf surface in biologically considerable amounts, which fluctuate between wide limits due to climatic conditions and the time of day. The investigation is still in progress.

The soil plays a major role as the source of minerals influencing metabolism of the green plant and the supply of phyllosphere nutrients exuded at the leaf surface.

DISCUSSIONS AND GENERAL CONSIDERATIONS

As the layer in contact with the leaf and with the atmosphere the phyllosphere is subject to the combined influences of both. From the former it derives its nutrients, the leaf exudates and leachings; from the latter it obtains water enriched by dissolved gases and salts. The developing phyllosphere population in turn exerts an influence on the underlying leaf by covering the surface, and by metabolic and autolysis products from the microbial population.

The nutritional status of the plant and, consequently, the mineral

and organic constituents of its leaf exudates are largely determined by the soil. The soil must therefore be included as a factor influencing the properties of the phyllosphere as an environment for the phyllosphere population. The whole community comprising the plant plus the population of micro-organisms of the phyllosphere is influenced by the environmental factors – light, temperature, and humidity – which show in addition to the seasonal variations diurnal fluctuations between physiologically considerable extremes. The daily variation in water supply, whether as rain, or condensed water vapour from soil and atmosphere, is the most important environmental factor for the phyllosphere vegetation. The exudates of the leaf and the soluble products of the phyllosphere population are dissolved and the survivors of the dry spell during the daytime are able to develop in this fresh supply of nutrients. On the other hand the leaf can then absorb this water to offset the losses by evaporation during the daytime. Along with the water, nutrients are taken up from the phyllosphere.

The intermittent exchanges between leaf and the phyllosphere result in an increase in the population of micro-organisms which gradually alters the conditions in the phyllosphere and leads through a slowly changing dynamic equilibrium to a climax in the microbial cover.

The occurrence of a microbial population rich in nitrogen-fixing organisms in the phyllosphere of a vegetation may be of decisive importance for the nutrition of that vegetation. Of the numerous papers in which some of the problems touching this field have been treated only a few will be mentioned in support of the above considerations.

1. *Water relations*

In a given edaphic zone and topographic site diurnal fluctuations in temperature and relative humidity within a vegetation are greatly influenced by the density and species composition of the plant community. The largest variations may be expected at the contact surface between the air and the vegetation. Here again the relief of the canopy, the form and the size of the individual plants modify spatially the pattern of the microclimate. Within a distance of a few meters, measured vertically or horizontally, considerable differences may occur. In a growing vegetation and expanding

canopy the microclimate in the phyllosphere shifts, therefore, from an outer zone with wide variations between complete wetting during a part of the day and complete desiccation during the rest of the time under insolation, to a not exposed region with lesser variations.

In the moist tropics the daily variations of the relative humidity of the atmosphere over the canopy may present a considerable range. Fluctuations from 40 to 94 per cent are common, and deposition of water may occur during most nights. Data on the actual incidence of dew are still scarce and vary considerably with the observation post. Ringoet [40] mentions an annual mean of 249 nights in Yangambi (Congo) with an absence during the rainy season. Zehnder [54] reports 254 nights for the hills covered with primeval forest of South Cameroon and 40 nights of dew fall in the savannah region of the north, whereas Schweizer [44] mentions 208 dew nights for Djember in East-Java. On mountain slopes and in the neighbourhood of extensive water surfaces, such as the sea, lakes or swamps deposition of water from the atmosphere may occur even more often: thus, Holttum [24] records nightly dewfall for Singapore. On the other hand Zehnder mentions an incidence of 150 dew nights on two stations on the coast. The quantities measured equal 0.2 to 0.3 mm of rain (Schweizer [44], Visser [51]). Comparing these recordings by the Leick and Duvdevani methods with those on a drosometer of his construction Masson [35] points out that the actual value may be easily 1.7 times higher.

The active transpiration during the day leads to a loss of water bringing the leaves to wilt, and necessitates an active intake of water to supply the deficit. According to Went [52] absorption of atmospheric water by the leaves begins when the relative humidity exceeds 93 per cent, a level which is regularly reached after the drop in temperature towards or during the evening. The amount of water absorbed by the leaves and transported to the plant body may be greater than the losses by evaporation during the day and part of it is then exuded again after some hours by the root or by the leaves. Thus the intake of water may become a major function of the leaf (Breazeale *et al.* [5, 6], Went [52]).

Variations in absorption of water by the leaves of different species due to the structure and the age, have been reported. For instance Ringoet in his study of the water balance of plantation crops mentions an increase of 10.9 per cent in the fresh weight of young

Elaeis leaves exposed to dew compared with 17.9 per cent in the case of old leaves. Such a quantity amounts to *ca* 10 litres of water per night for a tree having approximately 67 kg of leaves. For intact cacao plants the mean intake of dew water amounts to 8 per cent of the fresh weight of the leaves. Ringoet noticed, moreover, that the water absorption continues after sunrise. This is a point of interest, as it may be expected that due to the rising temperature the physiological activity of the phyllosphere population will increase, and that a steady uptake by the leaf of organic matter released in the medium by the microbes will continue until the water has completely disappeared. It forms subject for future research.

The variations in the wettability of the leaf surface in a diurnal rhythm, and in relation to the age of the leaf and the environmental factors have been the subject of a number of papers (Fogg [15 16], Linskens [31 32 33]). The decreasing wettability during the prematurity stage is apparently connected with the exudation of lipids and waxes at the leaf surface. The subsequent increase after the leaf has ceased to grow seems to be due to the continued excretion of organic acids and minerals and the resulting hydrolysis or saponification of the first formed wall constituents. Moreover the action of cutin-consuming micro-organisms may enhance the breakdown of the cuticle. The unabsorbed dew is the environment in which the phyllosphere population develops on the material excreted by and leached from the leaf. It appears of importance here, that the evaporation deficit of the day may cause an increased permeability of the leaf tissue, with the result that considerable amounts of minerals and organic matter are deposited on the leaf surface. According to Maximow [36] these are lost to the plant even when the wilting is reversible. It seems, however, more probable that in the humid tropics at least a part of such excretions may be re-absorbed.

2. *Nutrients in the phyllosphere*

The occurrence of a rich microbial population in the phyllosphere is in itself proof of an environment with a considerable, if not specific supply of nutrients; the abundance of epiphytes in the moist tropics or elsewhere under perhumid conditions – even if these only occur for part of the day – is another indication. In this con-

nection attention may be drawn to the Bromeliad *Tillandsia usneoides*, which covers the trees in the American tropics and subtropics, obviously living on plant excretions but which can also be selfsupporting on aerial wires. An exceedingly rich micropopulation has been observed on the leaves of this plant by the present author.

The data on the excretions of plant nutrients by leaves have vastly increased since Arens [1] and Lausberg [30] re-introduced the topic in 1934. The losses from field crops and trees due to leaching by rain are well known and have lately been studied in more detail both qualitatively and quantitatively. Recently renewed investigation into the inter-action between higher plants by way of excretion products has made clear the importance of a number of these substances (Grümmer [18]). In part they seem to be highly specific for particular plant species. The age of the leaf and its physiological condition at the time of or prior to the wetting determine the composition and the quantity of the exudate. It has been well established that nutrients are more readily leached from mature leaves and such approaching senescence (Arens [1], Tukey *et al.* [34 50]).

The exudates contain both mineral and organic materials, the latter sometimes being twenty times greater in quantity than the former. The losses by leaching may be severe; they are directly correlated with the leaching time. Tukey *et al.* [50] mention a loss of organic matter of 4.8 per cent of the dry weight of the leaves during 24 hours. With reservations for specific variations and the physical condition of the plant the losses amount from 0.5 to 7 per cent (Engel [14]) to 50 per cent of the ash content of the leaves (Arens [1], Lausberg [30]). Engel's lower figures may be explained by the fact that he rinsed the leaves before making the determinations and thereby removed part of the exuded materials.

The dry weight of the leaves in an established herbaceous vegetation may amount to 30 per cent of its total dry weight, and in a 18-year-old forest stand in the tropics still amounts to approximately 5 per cent (Bartholomew *et al.* [2]). It is clear, therefore, that daily considerable quantities of exudates are deposited on the leaf surface which are available as nutrients for the phyllosphere population. Washed off by rain they are incidentally returned to the soil. Dalbro [9] estimated in the temperate climate of Denmark

the amount of organic matter leached from apple trees to be more than one ton per hectare per annum, of potassium 23.3 to 28.3 kg, and of calcium 6.7 kg. Potassium, sulphates, and phosphates are easily removed by rainwater; this is supposed to play a role in the rapid turnover of nutrients in the vegetation (Hesse [23]).

Noteworthy are Schweizer's data on the mineral contents and sugars in dew and rainwater adhering to the leaf surface and in leachings of leaves of tropical plants. Whereas the ash content in rainwater varied from 66 to 188 mg/l for coffee, from 34 to 764 mg/l for tobacco, in dew the corresponding values varied from 294 to 410 mg/l and 180 mg/l, respectively. For both plant species the values for sugars lie within the range of from 8 to 19 mg/l in rainwater and 115 to 244 mg/l in dew; those for total nitrogen move between 9 and 19 mg/l and 25 and 80 mg/l, respectively. It seems possible that other reducing compounds are included under the heading of sugars, and that the value for nitrogen includes part of the nitrogen bound up in the phyllosphere population. The data are few and no mention is made of the time in the morning, when the dew was collected, a point which may be important in view of the high values obtained for sugar content. It has been ascertained by Tukey *et al.*[50] that the carbohydrate loss from leaves is directly correlated with light intensity and cumulative with the time of wetting. Keeping in mind that a very active microbial life has been observed by the present author in dew collected two hours after sunrise this would mean that towards the end of the wetting period a surplus of energetic material is still present in the milieu, and that the synthesis of microbial bodies may continue till the drying of the surface. Until the same time the uptake of material from the phylllosphere by the leaf will continue.

Schweizer's figures, however incomplete, show the order of variations to be expected, and support the opinion that the dew as such offers a richer medium for the maintenance of a phyllosphere population than rainwater. The few observations made by the present author agree with his data.

The diurnal variations in water content of the leaves has been mentioned above. It is suggested by the present author that nutrients accumulating on the outside of the leaf during the day may be subsequently re-absorbed together with the metabolic and decomposition products from the phyllosphere population during the

nightly wetting of the leaf surface. The results of leaf spraying experiments with radio-isotopes lend still more probability to the above contention (Thorne [48 49], Tukey *et al.*[50]). The intake proves to be most rapid during the first few hours after spraying, and continues during the entire period when free water is present on the leaf surface. Therefore, the duration of the wetting is doubly important, from the point of view of absorption of nutrients and water by the leaf as well as for the development of the phyllosphere population.

The water and nutrient status of the plant and its phyllosphere population thus appear to be closely interrelated. Dew is obviously more important than rain for the phyllosphere population as all the unabsorbed exudate remains available for its use. The regular rhythm of wetting and drying and the daily supply of nutrients in particular proportions from the leaf induces periodic increases of the micro-organisms which survive periods of drying and which are adapted to this particular kind of environment. As has been mentioned before a succession of organisms follow the gradual change in nutrient conditions resulting from the growing population and the changes in the physiological condition of the leaf.

Dew leads to an enrichment of the leaf environment whereas rain causes an overall loss due to leaching of the nutrients and run-off from the phyllosphere population. Persistent rains may be thus increasingly detrimental. The nutrients in the run-off may remain in the food cycle of the plant being taken up again by the root system, but likely to a lesser extent than by absorption by the leaf. Permanent losses from the site are apt to occur due to leaching and surface erosion of the soil.

What are the implications of the widespread occurrence in the tropics of the phyllosphere organisms, which for a great part are capable of fixing atmospheric nitrogen, on the leaves?

Primarily it suggests, as has been pointed out above, that the vegetation might profit by foliar uptake of the nitrogen bound by the microbial population of the phyllosphere in return for nutrients excreted by the leaves. More research is needed, however, to prove the correctness of the contention that a direct interrelation exists between the higher plant and the epiphyllic microbial flora. It has to be kept in mind that this relationship is different from that

in which symbiotic bacteria are located in special structures on the plant, *e.g.* root or leaf nodules.

Secondly it seems possible that the nitrogen in the soil is augmented by the nutrients and bacteria washed down from the living leaves.

Thus, it might be an additional explanation of the high nitrogen content of tropical soils beneath a plant cover and the recovery of soil fertility during cropped fallow following agricultural crops. The following considerations may be offered to elucidate the latter point.

The available-nitrogen status of tropical soils is a problem of longstanding importance, as lack of this nutrient is generally the factor preventing repeated annual cropping of the same site. It is now recognized that the exceeding richness of forest soil and of soils under vegetation cover in general, in organic matter and total nitrogen, prevails throughout the tropics, nothwithstanding the fact that the mineral status is often poor, *e.g.* equatorial Africa (Bartholomew *et al.*[2] Greenland[17]), Malaya (Corbet[8]), Indonesia (Hardon[20]), equatorial South America (Jenny[25]), *etc.* The total soil organic matter varies greatly with the type of vegetation, being highest under forest cover – either primeval, secondary or planted – and lowest in the case of bare soil. In between lie the soils under cultivation, with again considerable variation depending upon topography, soil type and structure, treatment received, type and duration of crop and climate. The C/N ratio of the soil organic matter generally varies between 10 and 20. The form in which nitrogen occurs is such, however, that it is not readily available as plant nutrient.

Apart from the combined nitrogen derived from rain, the gains in available soil nitrogen are ascribed to the results of nitrogen fixation by the Rhizobium-nodules of legumes, fixation of nitrogen by free-living bacteria and, finally, to decomposition of soil organic matter. Certainly nitrogen fixation in root-nodules may contribute an important amount especially in those regions where leguminous species are predominant. This is, however, decidedly not the case in all tropical vegetation. Moreover, the formation of nodules, if at all, and the occurrence of effective strains of Rhizobium seems to be exceedingly irregular (Bonnier[4]). Derx[11] suggests that the free-living soil bacterium Beijerinckia may provide the necessary combined nitrogen by association with tropical plant species, presumably legumes, without, however, excluding the non-legumes. The latter trend of thought is also found in Parker's[39] study of non-symbiotic nitrogen fixation in soils not supporting legumes. He emphasizes the importance of the association between higher plants and the potential nitrogen-fixing bacteria, living on root exudates and plant litter, as a major factor in the gains in fertility under grass leys. His argument, based on research in temperate regions, may easily be applied to tropical soils. Previously the importance of the storage of nitrogen in the soil by immobilization in microbial proteins has been stressed

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by Harmsen and Van Schreven [22]. In New Zealand Stevenson [46] has pointed out the gains of nitrogen in *Pinus radiata* plantations after 25 years of tree growth, where no other source than the atmosphere appears to be available for the observed accumulation in trees and in the soil. A similar example has been put forward for broad-leaf vegetation and here the accumulation is attributed by Stevenson to symbiotic fixation of nitrogen in the bacterial leaf nodules of members of the Rubiaceae. All these authors envisage the interaction on a large scale of the living organisms concerned.

Birch's [3] research on humus decomposition indicates that the largest proportion of available soil nitrogen is derived from humus, *i.e.* from dead plants. The drying and wetting cycle of the soil is all-important in the release of nitrogen. At the start of the wetting, *e.g.* under field conditions after rainfall, rapid mineralization sets in, which under persistently moist conditions declines within a few days to a lower and constant level, but a fresh release occurs on wetting after every drying. It is important that the degree of desiccation seems to determine the magnitude of the flush of nitrogen, the latter being proportional to the difference in soil moisture content before and after the wetting; moreover, it is also important that in each wetting cycle only a fraction of the nitrogen of the humus reserve is released and, therefore, the reserves persist. A similar rapid release of available nitrogen prior to the increase in bacterial numbers and due to a rapid mineralization after remoistening air-dried soils, has also been reported by Stevenson [47].

It explains the high level of ammonium and nitrate nitrogen in the soil under tree cover compared with that under fallow or rotation crop (Greenland [17], Hagenzieker [19], Hesse [23], and others) and also explains the flush of available nitrogen in the topsoil as the response to the wetting of the soil after rainfall.

All these considerations imply that the magnitude of plant growth, interrelated as it may be with the symbiotic and non-symbiotic fixation of nitrogen, eventually determines the amount of humus in the soil and is therefore a decisive factor in increases of soil fertility.

Dry-matter increments of 12,000 kg per ha per annum are not uncommon for forest stands. Regional variations in this value occur due to differences in vegetation type and environmental factors. The data of Bartholomew, Meyer and Laudelout [2] for forest fallow in Yangambi (Congo) will serve as an example of the order of these increments. Comparisons are made of increment in organic matter, estimated as total dry weight per hectare, and the contents of nitrogen and other plant nutrients in representative samples of leaves, stems *c.q.* wood, roots, dead wood and litter for secondary forest 2, 5, 8, and 18 years old. The trend of these increases for the different vegetative parts is shown in Fig. 2.

It is interesting that whilst the leaf weight remains constant there is a slight but steady increase in root weight and a spectacular increase in the amount of wood, which contains the bulk of the organic matter. The dry matter of the stand contains 701 kg of nitrogen per hectare, 80 per cent of

which had been accumulated within the first 5 years. In addition to the nitrogen locked up in the vegetation there are 1500 to 2500 kg per ha in the topsoil and 3000 to 5000 kg per ha in the layer of soil at 15 to 100 cm depth, for the greater part the product of the forest stand. In comparison, for a

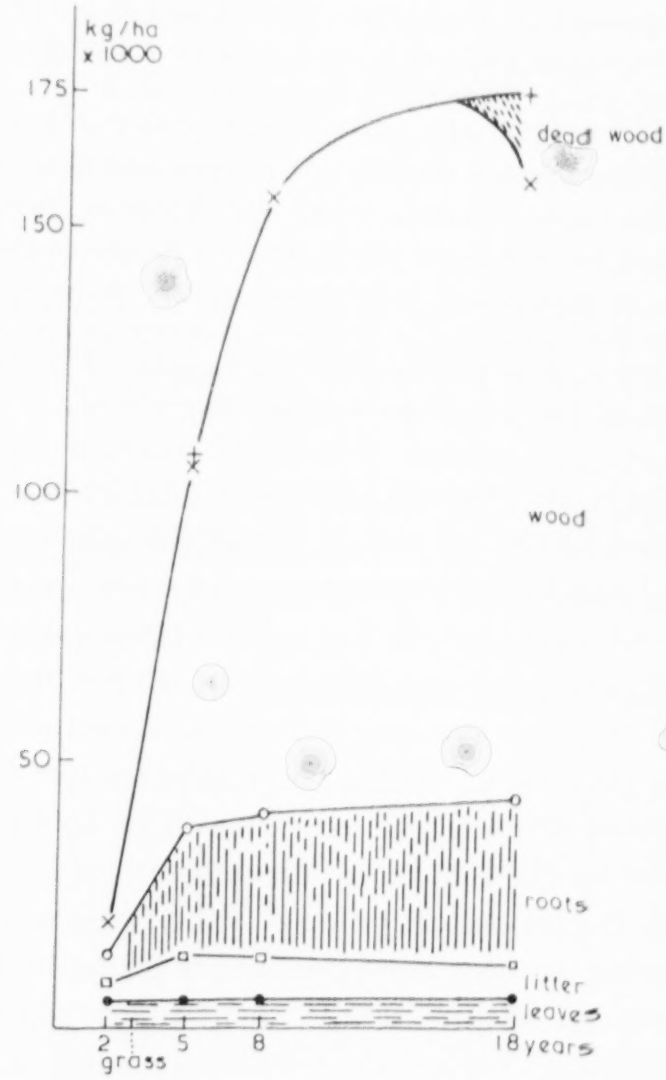

Fig. 2. Increases in dry matter of the vegetative parts of the plant cover in kg of dry weight per hectare, during 18 years of forest fallow in Yangambi (Congo). From the data of Bartholomew, Meyer and Laudelout [2].

Explanation: ● = leaf; □ = litter; ○ = roots; × = wood; + = dead wood.

cropped soil in the same region the corresponding values are from 1000 to 1500 kg in the topsoil and rapidly decreasing to 350 kg per ha in the layers from 15 to 100 cm depth.

The slow rate of decomposition of the organic matter in the soil under the prevailing conditions of high humidity makes it improbable that the nitrogen liberated in this process would be sufficient for the spectacular growth of the forest, It seems feasible that a more rapidly formed and,

equally, a more readily decomposed material, *i.e.* the cells of the micro-organisms, will play the deciding role in the supply of nitrogen to the plant community, either through the metabolic products excreted or by the decomposition of the microbial cells.

It has been established that in cultures of Azotobacter 10 to 25 per cent of the nitrogen present is extracellular (Burk and Horner[7], Roberg[41]); moreover, that in mixed cultures a greater efficiency of fixation may occur (*cf* Roberg). This would support the contention that the nitrogen-fixing bacteria initiate the process of microbial cell multiplication in the phyllosphere, with a follow-up of a number of other micro-organisms living on the excreted matter, and on the living or decomposing cells. Moreover, these products may be easily absorbed by the green plant. The constancy of the weight of the living leaves in an established vegetation appears to be significant in this connection since the foliage is the production centre of the organic material to be used on one side as plant-building substance or on the other side as the energy source of the phyllosphere population.

Summarizing it may be said that bacteria able to fix atmospheric nitrogen abound in the soil and in the phyllosphere; under suitable environmental conditions these organisms contribute to the supply of combined nitrogen for all the other organisms of the habitat. In the soil fully adequate conditions occur in root nodules. The close association of the two symbionts in these structures allows direct exchange of metabolic products. The role of the free-living nitrogen-fixing bacteria of the soil, however, is still in doubt and might be partly ascribed to the lack of an adequate supply of suitable energetic material. Increasing attention is being given to the rhizosphere population living on root exudates, apart from the bacteria living on the decomposition products of dead plant material, which as has been mentioned before are released in small amounts during short flushes after rewetting of the soil. It seems possible, however, that the big gains under grass leys as reported by Parker may be a joint effect due to the sugars exuded by the leaves and washed into the soil. In both these cases the higher plant is the ultimate source of energy material for the micro-organisms concerned. On the other hand in nitrogen-deficient soils the growth of micro-organisms and their nitrogen-fixing activities are recognized as essential for the survival of the plant cover, particularly so in the first stages of recolonization of bare soil.

A close association between micro-organisms and green plants also occurs in the phyllosphere. Here it is the transpiring leaf which supplies the necessary organic and mineral nutrients for the develop-

ment of the phyllosphere population. Free water as a major factor in this environment enables the phyllosphere population to use the leaf exudates and allows an exchange of materials to take place between the leaf and the phyllosphere. In this exchange proximity to the site of photosynthetic activity may prove to be highly advantageous. The diurnal rhythm of the variation in water supply controls excretion and release as well as absorption of materials. Variations in wetting, moreover, induce intermittent development and recession of part of the phyllosphere population during the wet and the dry periods respectively. From the intermittent flow of nutrients to and from the leaf, as well as from the phyllosphere, a steady nutritional state may result, which only gradually changes with the age of the leaf and the alterations in the composition of the microbial population. The readily decomposing microbial bodies provide directly available nutrients for both the higher plant and the phyllosphere population in short, daily flushes. The role of the free-living, nitrogen-fixing bacteria in this environment is likely to be of more consequence here than in the soil.

The discussed interrelations are schematically arranged in Fig. 3.

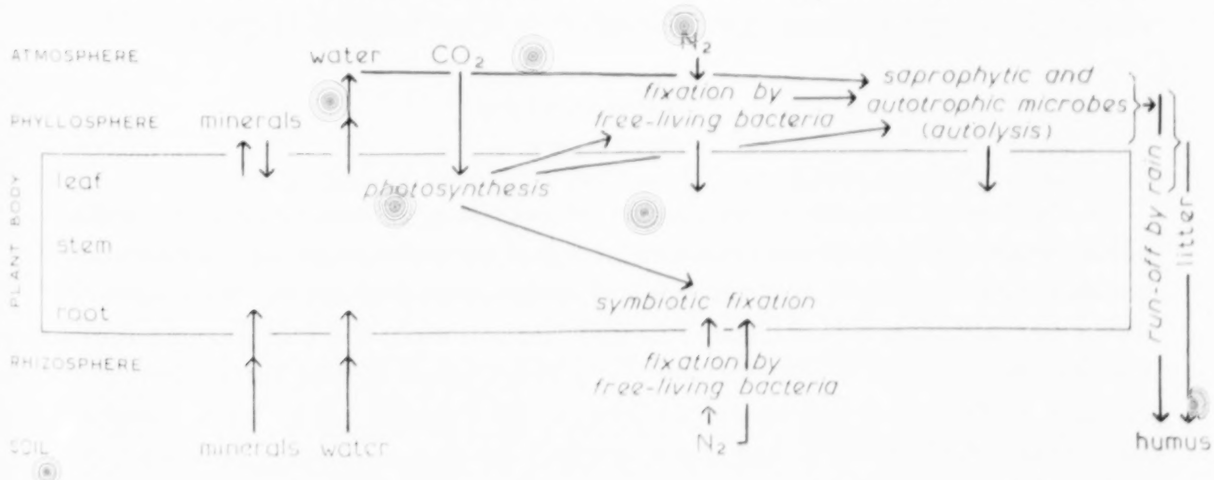

Fig. 3. Explanation in text.

It is obvious that the climate as a whole determines the existence of the phyllosphere population. The necessary conditions reach optimum levels in broad-leaf, evergreen forest of the moist tropics. The temperature is, generally, not a limiting factor. High relative humidity and daily wetting of the leaves either by rain, dew or condensed water vapour from the soil alternate with daily short periods of surface drying. The importance of these daily variations for the life of the higher plant and the phyllosphere population as

well as for the exchange of materials between the two has been emphasized. The production of organic matter by the vegetation is thus dependent on the supply of water.

This contention may account for the beneficial effect of plant cover on soil fertility and the rapid growth of tropical forest when once established, and also, for the relatively short periods of forest fallow allowable between two crops, as practised in shifting cultivation in the tropics. It might also be one of the reasons for the cultivation of tea, coffee and other tree crops, particularly in the early stages, under the shade of fast growing, albeit generally but not necessarily leguminous trees and also, for leaving the soil undisturbed and return to forest conditions, as advised by Harler [21] for the management of tea plantations. Eventually, it is in agreement with Willimott and Anthony's [53] suggestion for the South Sudan "that tree-crops in some form or other are the answer to the soil fertility–crop production problem".

Much research is needed to substantiate the above mentioned suggestions. They may, however, offer an alternative approach to the problems mentioned and open up a wider field of coordinated research in agriculture and forestry in the humid tropics.

SUMMARY

In the humid tropics a population of micro-organisms covers the foliage of the vegetation. It forms a continuous layer on the mature leaves and shows considerable variation in thickness and species composition on the upper and lower surface. A gradual change in distribution over the leaf surface and in species composition occurs during the physiological ageing of the leaves. It reaches a climax on the senescent foliage. By analogy with the rhizosphere, the term 'phyllosphere' has been applied to the micro-environment conditioned by the leaf.

Oligonitrophilic and nitrogen-fixing organisms abound in the phyllosphere. Among these, the representatives of the genera *Beijerinckia*, *Azotobacter*, *Aerobacter*, *Pseudomonas*, and *Spirillum* could be identified. They are the first organisms to colonize the leaf surface. The release of metabolic products from living and dying cells produces conditions which allow a succession of other organisms to develop.

The leaves retain a healthy green colour even under a considerable layer of micro-organisms unless unfavourable climatic conditions induce the ever-present semi-pathogens to infect the plant.

The phyllosphere as an environment is subject to influences from the whole plant as well as from the atmosphere, the changing population con-

tinually conditions it further. Climatic factors act directly as well as indirectly through the responses of the higher plant. Diurnal variations between physiologically considerable extremes are characteristic of the environment. Free water is essential for the phyllosphere organisms which consequently develop during the periods of wetting. Furthermore, water is the medium of exchange between the leaf and the phyllosphere population. Much depends upon the form in which the water is available, *i.e.* as rain or as dew. The duration of the wetting and the quantity of water determine the possibilities for development and survival of specific organisms in the wetting and drying cycle and, therefore, ultimately the species composition of the phyllosphere population.

The possible effects of this epiphyllic population on the growth of higher plants are discussed.

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